

We Claim:

1. An aqueous formulation comprising TFPI and a charged polymer wherein the concentration of TFPI is greater than 1 mg/ml.
2. The aqueous formulation of claim 1 wherein the concentration of TFPI is greater
5 than 5 mg/ml.
3. The aqueous formulation of claim 1 wherein the concentration of TFPI is greater
than 10 mg/ml.
4. The aqueous formulation of claim 1 wherein the concentration of TFPI is greater
than 20 mg/ml.
- 10 5. The aqueous formulation of claim 1 which is pharmaceutically acceptable.
6. The aqueous formulation of claim 1 wherein the charged polymer is a sulfated
polysaccharide.
7. The aqueous formulation of claim 1 wherein the charged polymer is heparin.
8. The aqueous formulation of claim 1 wherein the charged polymer is dextran
15 sulfate.
9. The aqueous formulation of claim 1 wherein the charged polymer is
polyphosphate.
10. A method of modifying the solubility of a protein having a first domain which
has a net positive charge and a second domain which has a net negative charge,
20 comprising the steps of:

adding to the protein an aqueous solution of a charged polymer to reduce
intermolecular or intramolecular interactions between the positively and negatively
charged domains.

11. The method of claim 10 wherein the first domain has a charge density of at least
25 five cationic amino acids in a series of ten consecutive amino acids.
12. The method of claim 10 wherein the first domain comprises five consecutive
cationic amino acids.
13. The method of claim 10 wherein the second domain comprises five consecutive
anionic amino acids.

14. The method of claim 10 wherein the second domain comprises five anionic amino acids in a series of ten consecutive amino acids.
15. The method of claim 10 wherein the protein is TFPI.
16. The method of claim 10 wherein the protein is a TFPI mutein.
- 5 17. The method of claim 10 wherein the protein is TFPI-2.
18. The method of claim 10 wherein the protein is in an insoluble form prior to the step of adding.
19. The method of claim 10 wherein a chaotropic agent is also added to the protein.
20. The method of claim 10 wherein the specific activity of the protein is increased
10 by said step of adding.
21. The method of claim 10 wherein the charged polymer is immobilized on a solid support.
22. The method of claim 10 further comprising:
applying the protein to a solid support before adding the charged polymer.
- 15 23. The method of claim 10 further comprising:
applying the protein to a solid support after adding the charged polymer.
24. The method of claim 22 wherein the solid support is an ion exchange resin.
25. The method of claim 23 wherein the solid support is an ion exchange resin.
26. The method of claim 20 wherein the protein is TFPI.
- 20 27. The method of claim 24 wherein the resin and the polymer have opposite net charges.
28. The method of claim 25 wherein the resin and the polymer have opposite net charges.
29. The method of claim 24 wherein the resin and the polymer have the same net
25 charge.
30. The method of claim 25 wherein the resin and the polymer have the same net charge.
31. The method of claim 24 wherein the charged polymer is added in a concentration gradient to effect selective elution from the solid support.

32. A method of refolding an improperly folded or denatured protein comprising the step of adding charged polymers to a solution comprising said protein prior to allowing said protein to refold.

33. The method of claim 32, wherein said polymer is a sulfated polysaccharide.

5 34. The method of claim 33, wherein said sulfated polysaccharide is dextran sulfate.

35. The method of claim 33, wherein said sulfated polysaccharide is heparin.

36. A method of refolding TFPI comprising the step of adding a charged polymer to a solution comprising improperly folded or denatured TFPI prior to allowing said TFPI to refold.

10 37. The method of claim 36, wherein the polymer is dextran sulfate.

38. The method of claim 36, wherein the polymer is heparin.

39. The method according to claim 38, wherein the heparin is added in solution.

40. The method according to claim 36 further comprising the steps of:
incubating said solution to allow said TFPI to refold, adding salt to
15 disassociate the polymer from the TFPI, passing the solution over an HIC column,
and recovering the TFPI.

41. A method of refolding TFPI comprising the step of immobilizing polymers of sulfated polysaccharides on a column and passing a solution of denatured TFPI through the column and eluting the refolded TFPI after the refolding has occurred.

20 42. The method of claim 41, wherein the sulfated polysaccharide is dextran sulfate.

43. The method of claim 41, wherein the sulfated polysaccharide is heparin.

44. A pharmaceutically acceptable composition comprising more than 0.2 mg/mL TFPI and a solubilizing agent, said solubilizing agent selected from the group consisting of: (a) acetate ion; (b) sodium chloride; (c) citrate ion; (d) isocitrate ion;
25 (e) glycine; (f) glutamate; (g) succinate ion; (h) histidine; (i) imidazole; and (j) SDS.

45. The composition of claim 44 wherein TFPI is present at a concentration of at least 1 mg/mL.

46. The composition of claim 44 wherein TFPI is present at a concentration of at
30 least 10 mg/mL.

47. The composition of claim 44 further comprising a secondary solubilizer, said secondary solubilizer selected from the group consisting of:
(a)polyethylene glycol; (b)sucrose; (c)mannitol; and (d)sorbitol.

48. The composition of claim 44 further comprising sodium phosphate at a
5 concentration greater than 20mM.

49. The composition of claim 44, wherein the composition is hypertonic.

50. The composition of claim 49 wherein the hypertonic composition comprises 0.5M NaCl.

51. The composition of claim 49 wherein the hypertonic composition comprises
10 0.5M NaPO₄.

52. The composition of claim 49 wherein the hypertonic composition comprises 0.5M sodium citrate.

53. The composition of claim 49 wherein the hypertonic composition comprises 0.5M sodium isocitrate.

15 54. The composition of claim 44 wherein the composition is isotonic.

55. The composition of claim 44 wherein the pH of the composition is below pH 7.0 and the solubilizer is not glycine.

56. The composition of claim 55 wherein the pH of the composition is pH 4.5 or below.

20 57. The composition of claim 44 wherein the solubilizer is acetate ion and the acetate ion is present in the composition as sodium acetate or potassium acetate at a concentration from 5 mM to 20 mM.

58. The composition of claim 44 wherein the solubilizer is sodium chloride and the sodium chloride is present in the composition at a concentration of at least 0.5M.

25 59. The composition of claim 44 wherein the solubilizer is citrate ion and the citrate ion is present in the composition as sodium citrate or potassium citrate at a concentration from 100 mM to 500 mM.

60. The composition of claim 44 wherein the solubilizer is isocitrate ion and the isocitrate ion is present in the composition as sodium isocitrate or potassium isocitrate
30 at a concentration from 100 mM to 500 mM.

61. The composition of claim 44 wherein the solubilizer is glycine and the glycine is present in the composition at a concentration from 5 mM to 20 mM.

62. The composition of claim 44 wherein the solubilizer is glutamate and the glutamate is present in the composition at a concentration from 5 mM to 20 mM.

5 63. The composition of claim 44 wherein the solubilizer is succinate ion and the succinate ion is present in the composition as sodium succinate at a concentration from 5 mM to 20 mM.

64. The composition of claim 44 wherein the solubilizer is histidine and the histidine is present in the composition at a concentration from 5 mM to 20 mM.

10 65. The composition of claim 44 wherein the solubilizer is imidazole and the imidazole is present in the composition at a concentration from 5 mM to 20 mM.

66. The composition of claim 44 wherein the solubilizer is sodium docecy1 sulfate and the sodium docecy1 sulfate is present in the composition at a concentration of 0.001 % to 0.1 % (weight / volume).

15 67. The composition of claim 47 wherein the secondary solubilizer is polyethylene glycol and the polyethylene glycol is present in the composition at a concentration of 0.2 % to 10 % (weight / volume).

68. The composition of claim 47 wherein the secondary solubilizer is sucrose and the sucrose is present in the composition at a concentration of 0.2 % to 10 % (weight /

20 volume).

69. The composition of claim 47 wherein the secondary solubilizer is mannitol and the mannitol is present in the composition at a concentration of 1.0 % to 5.0 % (weight / volume).

70. The composition of claim 47 wherein the secondary solubilizer is sorbitol and the

25 sorbitol is present in the composition at a concentration of 0.2 % to 10 % (weight / volume).